Fig 1

Murine MTN Blot
probed with ³²P-labelled
murine PDE_XIV

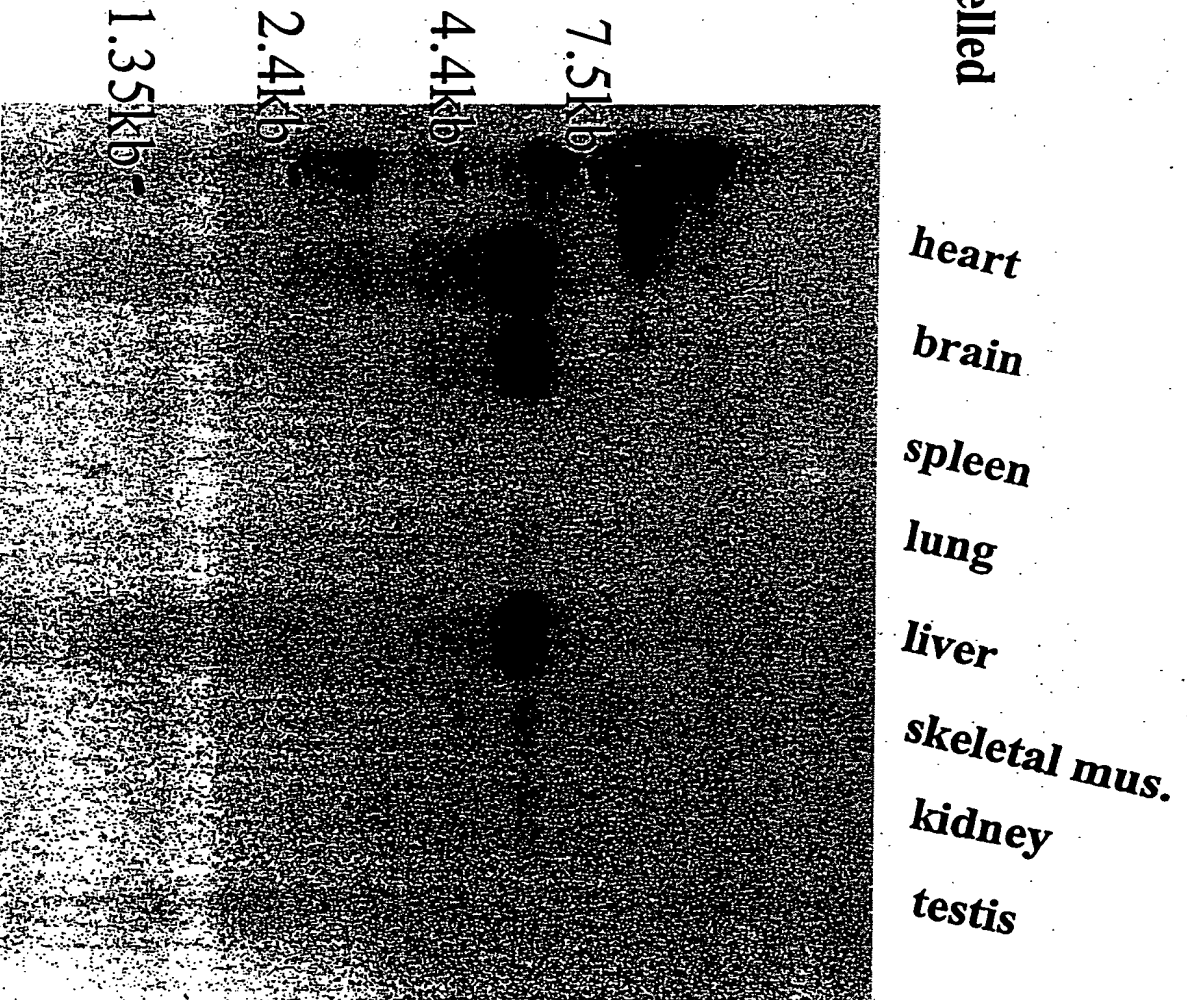7.5kb
4.4kb
2.4kb
1.35kb

heart

brain

spleen

lung

liver

skeletal mus.

kidney

testis

Fig 2

Murine Embryo MTN Blot probed with ^{32}P-labelled murine PDE_XIV
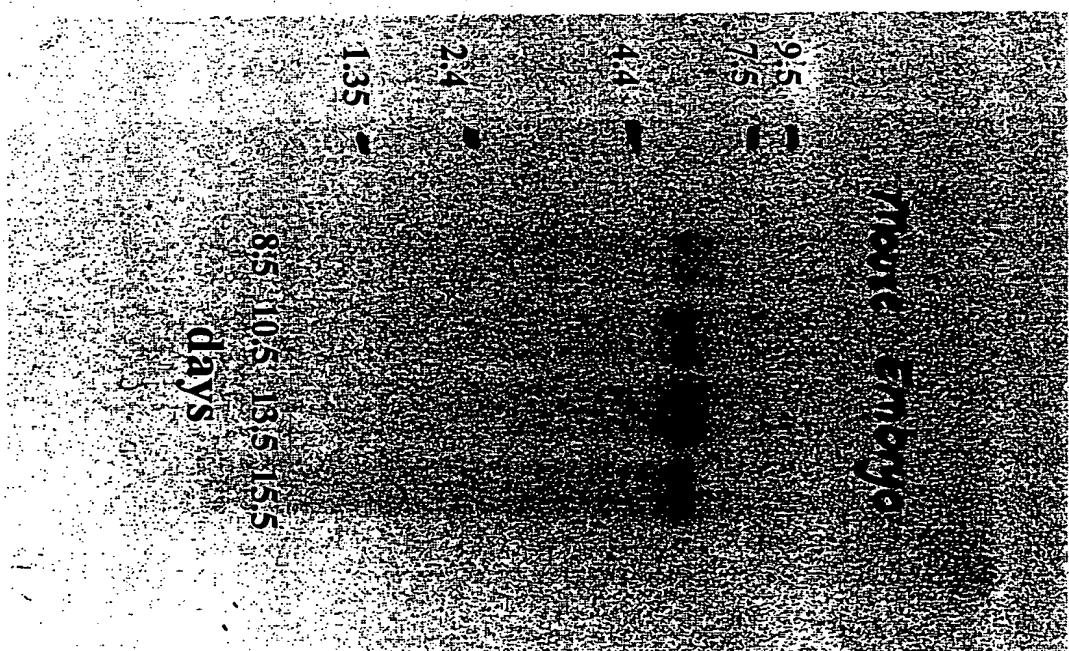
Figure 3

Human RNA Master Blot probed with ³²P-labelled
human PDE_XIV

|	1	2	3	4	5	6	7	8
A	whole brain	amygdala	caudate nucleus	cerebellum	cerebral cortex	frontal lobe	hippocampus	medulla oblongata
B	occipital lobe	putamen	substantia nigra	temporal lobe	thalamus	nucleus accumbens	spinal cord	
C	heart	aorta	skeletal muscle	colon	bladder	uterus	prostate	stomach
D	testis	ovary	pancreas	pituitary gland	adrenal gland	thyroid gland	salivary gland	mammary gland
E	kidney	liver	spleen	thymus	peripheral leukocyte	lymph node	bone marrow	
F	appendix	lung	trachea	placenta				
G	fetal brain	fetal heart	fetal kidney	fetal liver	fetal spleen	fetal thymus	fetal lung	
H	yeast total RNA 100 ng	yeast tRNA 100 ng	E. coli rRNA 100 ng	E. coli DNA 100 ng	Poly r(A) 100 ng	human C₀t1 DNA 100 ng	human DNA 100 ng	human DNA 500 ng
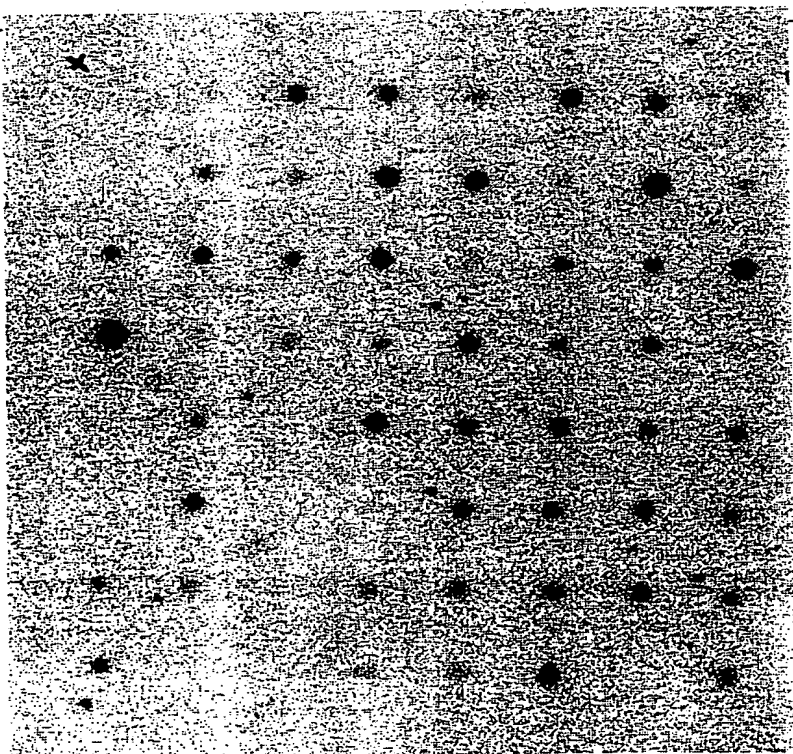
Fig. 4.   Alignment of the Murine and Human PDE_XIV nucleotide sequence

New sequence is PDE_XIV

Pileup: Genetics Computer Group.
    MSF: 3134   Type: N   Check: 5422   ..

Name: mpdea_ oo Len: 3134 Check: 5084 Weight: 0.001
Name: hspdea_ oo Len: 3134 Check: 338 Weight: 0.100

```
new mpde_        1 AGGTACGCCT GCAGGTACCG GTCCGGAATT CCCGGGTCGA CCCACGCGTC
new hspde_         .......... .......... .......... .......... ..........

new mpde_       51 CGGCCAGCCT CCCAGGCCGG CTGCCTGCTC ACCCAGCCAG TCGCTAGCTC
new hspde_       1 .......... .......... .......... .......... .CGGAAT.TC

mpdea_         101 TGGGCACTGC AGCAGGCTCG GCTCTGTCCC AGCGCTCGCT TGCTTGCTCG
hspde_           9 GATGCACTGC AGCAGGCTCG GCTCTGTCCC AGC....A.. ..........

mpdea_         151 CTCGCTCGGC TGGGAGAAAA GTGGTGTC.C TCGCCCAG.. AGAGCCTCTC
hspde_          43 ....CTTGTC TGGGAGAAAA GTGGTGTTAC TCACCCAGGG AGAGTCTCTC

mpdea_         198 TCTC..CCTT CCTTCTTTCT CGAGCTCTCT GAGTCCTTTG GCGTTTCTTT
hspde_          89 TTTCTACCTT CCTTCTTTCT CGATCTCCTT GTGTGCTTTT GTGTTTCTTT

mpdea_         246 CTTTCTTTCC TTTTTTTTTT TTTTTAATA TTTTCTTTTT CTTTCTATAA
hspde_         139 ATTTCTTTTC CTTTTTTTTC TT........ TTTTTTTTTT GTTACT....

mpde_          296 AACTTGCATA ATTATACTGC TAATCCTGGA TGAGGTTGCT GGATTCTGCA
hspde_         177 ........TA ATTATATTCC TAATCCTGGA TGAAGTTGCT GGATTCTGCA

mpde_          346 GCACAAATCT TCATGAACAA GCCGCACCGC TCAGAGATTT CACAGCATTC
hspde_         219 GCACAAGTCT TCATGAACAA GCAGCACCGC TCAGAGATTT CACGGCATTC

                                                      start codon
mpde_          396 AAAGGTCACA GAACTGCCAC TATGGTTAAA TGTCTTGTTT AATGGTTGAG
hspde_         269 AAAGGTCACA GAACTGCCAC TATGGTTAAA TGTCTTGTTT AATGGTTGAG

mpdea_         446 AGGTGTGGCG AAGTCTTGTT TGAGAGCCCT GAACAGAGTG TCAAATGTGT
hspde_         319 AGGTGTGGCG AAATCTTGTT TGAGAACCCC GATCAGAATG CCAAATGTGT

mpde_          496 TTGCATGCTA GGAGATGTAC GACTAAGGGG TCAGACGGGG GTTCCTGCCG
hspde_         369 TTGCATGCTG GGAGATATAC GACTAAGGGG TCAGACGGGG GTTCGTGCTG

mpde_          546 AACGCCGTGG CTCCTACCCA TTCATTGACT TCCGTCTACT TAACAATACA
hspde_         419 AACGCCGTGG CTCCTACCCA TTCATTGACT TCCGCCTACT TAACAGTACA

mpde_          596 ACACACTCAG GGGAAATTGG CACCAAGAAA AAGGTGAAAC GACTGTTAAG
hspde_         469 ACATACTCAG GGGAGATTGG CACCAAGAAA AAGGTGAAAA GACTATTAAG

mpde_          646 TTTCCAAAGA TACTTCCATG CATCTAGGCT TCTCCGGGGG ATTATACCGC
hspde_         519 CTTTCAAAGA TACTTCCATG CATCAAGGCT GCTTCGTGGA ATTATACCAC

mpde_          696 AGGCCCCTCT CCACCTGCTG GATGAAGACT ACCTTGGACA AGCAAGGCAC
hspde_         569 AAGCCCCTCT GCACCTGCTG GATGAAGACT ACCTTGGACA AGCAAGGCAT

mpde_          746 ATGCTCTCCA AAGTTGGAAC GTGGGACTTT GACATTTTCT TGTTTGATCG
hspde_         619 ATGCTCTCCA AAGTGGGAAT GTGGGATTTT GACATTTTCT TGTTTGATCG
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        mpde_    796 CTTGACAAAT GGGAACAGTC TGGTAACTCT GTTGTGTCAC CTCTTCAACT
        hspde_   669 CTTGACAAAT GGAAACAGCC TGGTAACACT GTTGTGCCAC CTCTTCAATA

 5      mpde_    846 CCCATGGGCT CATCCACCAT TTCAAGCTCG ATATGGTGAC CTTGCACAGG
        hspde_   719 CCCATGGACT CATTCACCAT TTCAAGTTAG ATATGGTGAC CTTACACCGA

        mpde_    896 TTTCTGGTTA TGGTTCAGGA AGATTACCAC GGTCACAACC CATACCACAA
        hspde_   769 TTTTTAGTCA TGGTTCAAGA AGATTACCAC AGCCAAAACC CGTATCACAA
 10
        mpde_    946 TGCTGTTCAC GCAGCCGACG TCACCCAGGC CATGCACTGT TACCTGAAGG
        hspde_   819 TGCTGTTCAC GCAGCCGACG TCACCCAGGC CATGCACTGC TACCTGAAAG

        mpde_    996 AGCCAAAGTT GGCAAGCTTC CTCACACCTC TGGACATCAT GCTTGGACTA
 15     hspde_   869 AGCCAAAGCT TGCCAGCTTC CTCACGCCTC TGGACATCAT GCTTGGACTG

        mpde_   1046 CTGGCTGCAG CAGCTCATGA CGTGGACCAC CCAGGGGTCA ACCAGCCATT
        hspde_   919 CTGGCTGCAG CAGCACACGA TGTGGACCAC CCAGGGGTGA ACCAGCCATT

 20     mpde_   1096 TTTGATCAAA ACTAACCACC ATCTTGCCAA CCTGTATCAG AATATGTCTG
        hspde_   969 TTTGATAAAA ACTAACCACC ATCTTGCAAA CCTATATCAG AATATGTCTG

        mpde_   1146 TACTGGAGAA TCACCACTGG CGATCTACAA TTGGCATGCT TCGAGAATCA
        hspde_  1019 TGCTGGAGAA TCATCACTGG CGATCTACAA TTGGCATGCT TCGAGAATCA
 25
        mpde_   1196 CGGCTCCTGG CTCACTTGCC AAAGGAAATG ACACAGG... .....ATATC
        hspde_  1069 AGGCTTCTTG CTCATTTGCC AAAGGAAATG ACGTAAGTGC TGCCGAGATG
                                                     stop codon

 30     mpde_   1238 GAACA..... ....GCA... ......GCTG GGCTCCCTCA TCTTGGCCAC
        hspde_  1119 AAACATACTG ATGTGCATGC AGTAAAGATA AGCCACTTTC TCTAGGGCA.

        mpde_   1270 GGATATCAAC AGACAGAATG AGTTTCTGA. ......CCCG CTTAAAAGCT
        hspde_  1168 GGCTTGGGAC CTTTTGCGTG AATGGCAGAG AGCCCCCCGG CTGTACTTCC
 35
        mpde_   1313 CACCTCCACA ATAAAGATT. TGAGAC.... ..TGGAGAAT GT.ACAGGA.
        hspde_  1218 TGCCTGCACT GAGCTGTCTA TCAGAGGAGA TTTGGTGTCA GTTACAGCAA

        mpde_   1354 ..CAGACACT TTATGCT.TC AGATCGCCTT GAAGTGTGCT GACATTTGCA
 40     hspde_  1268 CCCAGAAACC AAAATCTCTC TGTGTGCTTT GAAAGGGCCT TGCAGAGTCA

        mpde_   1401 AT..CCTT.. GTC.GTATCT GGGAGATGAG CAAGCAGT.. GGAGTGAAAG
        hspde_  1318 ATGACCTACA GTCAGGAAAA GGGATAATAA ACAGCTCTCA GTTTTCACAC

 45     mpde_   1444 GGT....... CTGTGAGGAA TTCTACAGAC AAGGTGACCT TGAAC..AG.
        hspde_  1368 GCTTCAGTAT CAGTGCTCAA CTTTGCCAAA TTCCCGACCT TTAGTTTAGC

        mpde_   1484 AAGTTTGAAC TGGAAATCAG .TCCTCTTTG TAATCAAC.A GAAAGATTCA
        hspde_  1418 AAAATTGTCC TTCCATGTAG CTCCAAATAG TAAATATTTA TCAAGAAGGA
 50
        mpde_   1532 ATCCCTAGCA TACAAA...T TGGTTTCATG ACT.TACATC GTGGAGCCGC
        hspde_  1468 A.CCCAGGCA TTCTAAAGCT AGAGTTCAAA AAAGTATATT TTGTAATTGC

        mpde_   1578 TGTTCCGGG. ...AGTGG.. GCCCGGTTTA CTGGG..AAC AGCACCCTGT
 55     hspde_  1517 TAGTCTCAGC AAAAATAGAA GTCAGAAATT CTTTTCTAAA ATGTCTTTTG

        mpde_   1620 CGGAGAACAT GCTAAGCC.. ....ATCTCG CGCACAACAA AGCCCAGTGG
        hspde_  1567 CTAAGTAATT GAAATGGCCC TAGCATTTTT TTCACCAATT AATTTACCTT

 60
        mpde_   1664 AAGAGCCT.G CTGTCCAATC AGCAC...AG ACGCA..... ...GGGGCAG
        hspde_  1617 ACGTCTCTTG CACTTTAAAC AGAAGGGGAG ACACTCATTT TCTGGTTCAC

        mpde_   1702 CG.......G CCAGGACCTG GCGG....GC CCCGC...AC CTGAGACCCT
 65     hspde_  1667 TATTTGATAG CCATGGTATG TAGGCTGAGT CCCACTAAAT CTGAGGCCAT

                                          stop codon
        mpde_   1738 GGAG.CAGAC AGAAGGTGCC ACGCCCTAAG GTAGCTGTC. .TGCTGA..T
        hspde_  1717 TGTTTCATTT TCCTGGTG.. ..GCCCCAAG TTAGCTGCTA ATACTGTCTT
 70
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      PC10315AGPR
      mpde_      1783 GCACGGCCA. .......TCT G.TCCGTCCA .....CAGGA GCACGGCC..
      hspde_     1763 CCAAGGCCAC CATTAATTCT GATCTGTTTA ATGAACACGT GCAGAACCCA

      mpde_      1817 ...ATCC... .........G TCC...GACT GC........ .CCTCGCAAC
   5  hspde_     1813 AGAAACCTAG GTGAAAAGAG TACATAGATT GCTGTACCCT TCTTCAAGAC

      mpde_      1840 AAGCCCATCA CGCTGGGTTT CGATGCCAT. .CCGCCTGCC A.CTTACC..
      hspde_     1863 AAGCACATAA CTTGAGGTCA AGGACCAAGT GCTGTCTCCC AACTGAACAA

  10  mpde_      1885 ...GCCTCCC TTCGTTGATC CAAGTGTACA AAAGCCATTG ...TCACCTC
      hspde_     1913 GCAGTATACT CTGGGTTGTG GATTGATTCC TGGCCCTCTG ATTTGATCTC

      mpde_      1929 AGCAT..... .TAGCTGCC. ..GAAATGGG CGGCTCTATC CCGTCATTGG
      hspde_     1963 ATGCTGTTTC CTAGCACCCA GAGGAATGTG AAATTTGCAG GAGGAATTTC
  15
      mpde_      1970 AG..CTGAT. ...TCTGGGG CGGCTGCCCC AACCGAAAC. .........G
      hspde_     2013 AGTTCTGATA AATTTTTACT CCCTGGAACT AAATAAAACC AGTTCTCGTG

      mpde_      2004 CCTGGAAGTA AGAA..AGGG GTGCTTCTGC CGTGTTCGCC TCTGGCCCTT
  20  hspde_     2063 CATGGAATAA AAACTTATGC CTCTTACTAG AATAATAAAT TGCAAAGATT

      mpde_      2052 GGTCACGCTG ACTGGCAGTA GCTCCTAAGT CCAGAGCATT TTAACGTTTG
      hspde_     2113 GAAAGAATTA AATGCAAAAA GAACTAAAAA CTAGAGCAAA AGATCAAGTG

  25  mpde_      2102 CCATC..GGA CAGCTGACCT ...GCATGAC ACCAGCAT.. .ACTTGGAAC
      hspde_     2163 AGAAGAAGAA AAGAGGAGGT AAGGAGAGAG ACAAGGAAGA AAGAAGGAGA

      mpde_      2144 TGCAAAACTG GTCTTGCGTG CCAGAGCACA AACGAGAGTG TGAGAGAAA.
      hspde_     2213 AGGAAAGGAA GAATAGTGAG G.ACAGGAAA GAAGAAAATG CAAGGGAAAT
  30
      mpde_      2193 ..GTACCTTC TATTT..TAA TAATAATTAT TATTATAAAA TA....ATAA
      hspde_     2262 GGGAAAGGAC TCTGGGGTGA CCAGACTTCT CCTGGTCAGT ACCTGCATTC

      mpde_      2235 ATCTTTTTAA CTTTT..ATA TTTCATGCAC CAGACAATGG GTCTAAAACT
  35  hspde_     2312 ATCCTGTTTG TTACTCAATA TTTCTTTCCT AAAATATTCA TTTCACATCT

      mpde_      2283 TTGGA...CA AGTAATACTC TGCGTACCCA AACCTAAGAG G......GGG
      hspde_     2362 ATGGATTCCA ATGAAAAATA TATTTTTATG TGTCTTTGTG GAACACAGTG

  40  mpde_      2324 TTC...ATTA TTTT.GCTAT T.GACTC... ..TATGCCAC ATTGGGTCCG
      hspde_     2412 TTATAAATTG TTTTTGCCAG AAGAATAATT GTTATACAAT AATATATGTG

      mpde_      2364 AGA..TGTGG CACCATTGCG ATTTCTGAAA CCACGCGTCC .CCTCCCATC
      hspde_     2462 AAAACTTTAT TACAAAGCC ATTATCATAA TCATTATTAT TCCTTCTATC
  45
      mpde_      2411 TGGTGGAAGG TGCTGTACAG CCCGTCCC.. ..TTTGCACC GTTAGCCAAT
      hspde_     2512 ACA.GGTAAA TGCTTTAATG TCATTTTTCT GATTTAAAA GTAGGGCAGG


  50  mpde_      2457 CCGTCTTT.. ....TACGGA ...TTCAGTG ACCTGTTTAT ATTCACAA.G
      hspde_     2561 TTAATTGTAG AAAGTAAGGA AAATTCAGGA AAGTGTTAGT TTGAACTATG

      mpde_      2497 TGTACATTTT CTGT..AAAT ACCAAACGCT ACTGA..... ......TTCC
      hspde_     2611 TGAAGTTGCT CTTTTTAAGG GCCAAAACA GGAGACTTTT AGCACTTTCA
  55
      mpde_      2534 CATGC..CA. ....AAATAC ACGAGTATTA TGGGATTGCT A.....CCTG
      hspde_     2661 TATGTTTCAG CTTGATATGA AAGAGAAAC TGAAACTGCT AGTAATCCTG

      mpde_      2571 .........T ATAAACAATG GCACTGTGAA CAGAATA... .CTGTTAGTT
  60  hspde_     2711 CCATCCAGGT ATAGTTCATG TTAACCTGGC TAGTTTATTT TCTTTTAGTC
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PC10315AGPR

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      mpde_   2608 TTAATACAAG AGAATGCATT TGTAAATATG GTATAGAGTT TATTAATATA
      hspde_  2761 TTTTTTCAAT ACAAA.CTTA TTTTAACAAA ATAT.GATTA TATTTGGGGA

 5    mpde_   2658 CTGTTGTTCG CAGATAAAGG CCTTAACTTT AAAAAAAAAA AAAAAAAAA.
      hspde_  2809 ACTTATTTTA CAGTTTACGT CCTGAAATTT TTTATTTACA ATAAAGACTT

      mpde_   2708 ......AAAA AAAAAAAAAA AAAAAGGGGC GGCCGCTCTA GAGGATCCCT
      hspde_  2859 TTTTCCAAAT CAAAAAAAAA AAAAAGGGGC GGCCGCTCTA GAGGATCCCT
10
      mpde_   2752 CGAGGGGCCC AAGCTTACGC GTGCATGCGA CGTCATAGCT CTCTCCCTAT
      hspde_  2909 CGAGGGGCCC AAGCTTACGC GTGCATGCGA CGTCATAGCT CTCTCCCTAT

      mpde_   2802 AGTGAGTCGT ATTATAAGCT AG........ .... 2823
15    hspde_  2959 AGTGAGTCGT ATTATAAGCT AGGCACTGGC CGTC 2992
```

Figure 5.   **Protien Alignment of the Murine & Human PDE_XIV**

CLUSTAL W (1.7) multiple sequence alignment

```
mpde    MSCLMVERCGEVLFESPEQSVKCVCMLGDVRLRGQTGVPAERRGSYPFIDFRLLNNTTHS 60
hspde   MSCLMVERCGEILFENPDQNAKCVCMLGDIRLRGQTGVRAERRGSYPFIDFRLLNSTTYS 60
        ***********:***.*:*..*******:******* ****************.**:*

mpde    GEIGTKKKVKRLLSFQRYFHASRLLRGIIPQAPLHLLDEDYLGQARHMLSKVGTWDFDIF 120
hspde   GEIGTKKKVKRLLSFQRYFHASRLLRGIIPQAPLHLLDEDYLGQARHMLSKVGMWDFDIF 120
        **************************************************** ******

mpde    LFDRLTNGNSLVTLLCHLFNSHGLIHHFKLDMVTLHRFLVMVQEDYHGHNPYHNAVHAAD 180
hspde   LFDRLTNGNSLVTLLCHLFNTHGLIHHFKLDMVTLHRFLVMVQEDYHSQNPYHNAVHAAD 180
        ********************:**********************.:**********

mpde    VTQAMHCYLKEPKLASFLTPLDIMLGLLAAAAHDVDHPGVNQPFLIKTNHHLANLYQNMS 240
hspde   VTQAMHCYLKEPKLASFLTPLDIMLGLLAAAAHDVDHPGVNQPFLIKTNHHLANLYQNMS 240
        ************************************************************

mpde    VLENHHWRSTIGMLRESRLLAHLPKEMTQDIEQQLGSLILATDINRQNEFLTRLKAHLHN 300
hspde   VLENHHWRSTIGMLRESRLLAHLPKEMT-------------------------------- 268
        ***************************

mpde    KDLRLENVQDRHFMLQIALKCADICNPCRIWEMSKQWSERVCEEFYRQGDLEQKFELEIS 360
hspde   ------------------------------------------------------------

mpde    PLCNQQKDSIPSIQIGFMTYIVEPLFREWARFTGNSTLSENMLSHLAHNKAQWKSLLSNQ 420
hspde   ------------------------------------------------------------

mpde    HRRRGSGQDLAGPAPETLEQTEGATP 446
hspde   --------------------------
```

Zinc binding motif's are highlighted in bold.
Non-Catalytic domain in italics.

Figure 6.

SPA assay to determine the cAMP hydrolytic activity of murine and human PDE_XIV.

percentage activity

protein concentration

—□— n. mouse
—△— MOCK
—○— n. human